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**PRODUCTION OF REACTIVE OXYGEN SPECIES BY POLYHALOGENATED
CYCLIC HYDROCARBONS (PCH)**

by

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SUMMARY

During the past three years, excellent progress has been made relative to the specific objectives of this project. The results strongly support the hypothesis that the ability of polyhalogenated cyclic hydrocarbons (PCH) to induce production of reactive oxygen species may contribute to many of the toxic manifestations associated with these xenobiotics. As a consequence of the studies, seven papers have been presented at national and international meetings. In addition, twelve manuscripts have been published, three manuscripts are in press, and one manuscript has been submitted. Furthermore, several manuscripts are in the process of being written. Copies of the reprints and manuscripts are appended.

A non-invasive method for assessing lipid peroxidation and oxidative stress has been developed. This method involves the simultaneous determination of the peroxidative lipid urinary metabolites formaldehyde, malondialdehyde, acetaldehyde and acetone by HPLC. These techniques have many potential applications. Structurally dissimilar xenobiotics as TCDD, carbon tetrachloride, paraquat, menadione, and alachlor induce the excretion of these lipid metabolites. These compounds represent xenobiotics with dissimilar mechanisms for inducing an oxidative stress and enhanced lipid peroxidation.

We have demonstrated that PCH can induce the formation of reactive oxygen species both in vitro and in vivo. PCH as endrin and lindane can induce formation of reactive oxygen species directly, and may not involve an indirect, hormonal or messenger system. These PCH can induce formation of reactive oxygen species in peritoneal macrophages, mitochondria and microsomes. Thus, multiple sources of reactive oxygen species exist in response to PCH.

The ability of antioxidants to attenuate the biochemical consequences of endrin-induced oxidative stress has been demonstrated in vivo. The administration of vitamin E succinate and ellagic acid to rats significantly modulates the formation of DNA single strand breaks, the increased hepatic lipid peroxidation, and the increased urinary excretion of lipid metabolites following an acutely toxic dose of endrin.

A relationship appears to exist between the ability to induce reactive oxygen species and the formation of oxidative tissue damage including lipid peroxidation, DNA single strand breaks, and decreased membrane fluidity. In addition, PCH induce altered calcium and iron homeostasis. The altered calcium distribution may lead to irreversible cell death, while altered iron distribution may contribute to the formation of reactive oxygen species.

Malondialdehyde is extensively used as an index of lipid peroxidation. Interest in malondialdehyde arises from the fact that it reacts readily with the functional groups of many cellular compounds. Following the administration of malondialdehyde to rats, a large increase in the excretion of both malondialdehyde and acetaldehyde occur. Furthermore, a new urinary metabolite, methyl ethyl ketone, is formed. The results demonstrate that orally administered malondialdehyde is rapidly excreted in the urine, and alters the metabolism and excretion of other lipid metabolites.

All of the specific aims presented in the proposal have been addressed by the investigators. The results have significantly contributed to our knowledge regarding the roles of reactive oxygen species in the toxicity of a wide variety of structurally dissimilar polyhalogenated cyclic hydrocarbons.

I. PRESENTATIONS AT SCIENTIFIC MEETINGS

Excretion of Formaldehyde, Acetaldehyde, Malondialdehyde and Acetone in the Urine of TCDD-Treated Rats. M.A. Shara and S.J. Stohs. Society of Toxicology, 1991. Dallas, Texas.

In Vitro Induction of Reactive Oxygen Species by 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Other Polyhalogenated Cyclic Hydrocarbons in Peritoneal Macrophages, and Hepatic Mitochondria and Microsomes. M. Bagchi and S.J. Stohs. 11th International Symposium on Chlorinated Dioxins and Related Compounds, 1991. Research Triangle Park, North Carolina.

Endrin-Induced Oxidative Stress in Female Sprague-Dawley Rats. M. Bagchi, E.A. Hassoun, D. Bagchi and S.J. Stohs. Society of Toxicology, 1992. Seattle, Washington.

The Effects of Anti-TNF- α Monoclonal Antibody and Dexamethasone on TCDD-Induced Oxidative Stress in Mice. N.Z. Alsharif, E. Hassoun, M. Bagchi and S.J. Stohs. Society of Toxicology, 1992. Seattle, Washington.

Excretion of Malondialdehyde, Acetaldehyde and Acetone in the Urine of Rats in Response to an Acute Dose of Malondialdehyde. P.I. Akubue and S.J. Stohs. Society of Toxicology, 1992. Seattle, Washington.

Comparative Effects of Endrin on the Hepatic Lipid Peroxidation, DNA Damage, and Nitric Oxide Production by Peritoneal Lavage Cells from C57Bl/6J and DBA/2 Mice. M. Bagchi, E.A. Hassoun, P. Akubue and S.J. Stohs. Society of Toxicology, 1993. New Orleans, Louisiana.

Protective Effects of Antioxidants Against Endrin-Induced Hepatic Lipid Peroxidation, DNA Damage, and Excretion of Urinary Lipid Metabolites. D. Bagchi, E.A. Hassoun, M. Bagchi and S.J. Stohs. Society of Toxicology, 1993. New Orleans, Louisiana.

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7. Bagchi, D., Dickson, P.H. and Stohs, S.J. The Identification and Quantitation of Malondialdehyde, Formaldehyde, Acetaldehyde and Acetone in Serum of Rats. Toxicol. Methods **2**, 270-279 (1992).
8. Bagchi, D., Bagchi, M., Hassoun, E.A. and Stohs, S.J. Time-Dependent Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) on Serum and Urine Levels of Malondialdehyde, Formaldehyde, Acetaldehyde and Acetone in Rats. Toxicol. Appl. Pharmacol. (in press).
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16. Akubue, P.I., Bagchi, D., Ihm, W.J. and Stohs, S.J. Excretion of Malondialdehyde, Formaldehyde, Acetaldehyde, Acetone and Methyl Ethyl Ketone in the Urine of Rats Given an Acute Dose of Malondialdehyde. (submitted).

III. IDENTIFICATION AND QUANTITATION OF LIPID METABOLIC PRODUCTS IN RESPONSE TO POLYHALOGENATED CYCLIC HYDROCARBONS (PCH)

One of our specific aims is the assessment of tissue damage in response to oxidative stress induced by polyhalogenated cyclic hydrocarbons (PCH). Lipid peroxidation is a potentially useful method for assessing oxidative damage, but in the past, a highly specific, non-invasive method for assessing peroxidative damage has not been available. We have developed methods for the simultaneous identification of formaldehyde, acetaldehyde, malondialdehyde and acetone in urine and serum of experimental animals, and have quantitated the urinary excretion of these lipid metabolites in response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), endrin, alachlor, menadione, carbon tetrachloride and paraquat. In addition, we have also quantitated the serum levels of these metabolites with time in response to TCDD.

Formaldehyde (FA), acetaldehyde (ACT), malondialdehyde (MDA) and acetone (ACON) were simultaneously identified in urine, and their excretion quantitated in response to chemically induced oxidative stress. Urine samples of female Sprague-Dawley rats were collected over dry ice and derivatized with 2,4-dinitrophenylhydrazine. The hydrazones of the four lipid metabolic products were quantitated by high-performance liquid chromatography on a Waters 10- μ m μ -Bondapak C₁₈ column. The identities of FA, ACT, MDA and ACON in urine were confirmed by gas chromatography-mass spectrometry. An oxidative stress was induced by orally administering 100 μ g/kg 2,3,7,8-tetrachlorodibenzo-p-dioxin, 75

mg/kg paraquat, 6 mg/kg endrin or 2.5 ml/kg carbon tetrachloride to rats. Urinary excretion of FA, ACT, MDA and ACON increased relative to control animals 24 hrs after treatment with all xenobiotics. The system has wide-spread applicability to the investigation of altered lipid metabolism in disease states and exposure to environmental pollutants.

A high pressure liquid chromatographic (HPLC) method has been developed for the simultaneous determination of serum levels of malondialdehyde (MDA), formaldehyde (FA), acetaldehyde (ACT) and acetone (ACON). Serum samples were derivatized with 2,4-dinitrophenylhydrazine (DNPH) to form the corresponding hydrazones of the four lipid metabolites. The hydrazones were extracted with pentane and chromatographed on a μ Bondapak C₁₈ column. Acetonitrile-water (49:51 v/v) was used as the mobile phase. Treatment of rats with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (50 μ g/kg) resulted in marked time-dependent increases in the serum levels of the four metabolites. This HPLC method for identifying and quantitating formaldehyde, malondialdehyde, acetaldehyde and acetone is rapid and highly reproducible. The method has widespread applicability to the assessment of metabolic alterations produced by drugs, disease states and toxicants.

IV. TIME-DEPENDENT EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON SERUM AND URINE LEVELS OF MALONDIALDEHYDE, FORMALDEHYDE, ACETALDEHYDE AND ACETONE IN RATS

TCDD was used as a prototypical PCH to assess the comparative effect of a chemically induced oxidative stress on serum and urine levels of lipid metabolites. The ability of TCDD to produce lipid mobilization and alter lipid metabolism is well known. Previous studies have indicated that TCDD induces an oxidative stress and enhances lipid peroxidation. However, the products resulting from altered lipid metabolism in response to TCDD have not been studied. We have examined the time-dependent changes in serum and urine levels of malondialdehyde, formaldehyde, acetaldehyde and acetone in response to a single oral 50 μ g/kg dose of TCDD in rats. The changes in these four metabolic products were quantitated by high pressure liquid chromatography (HPLC). The effects of TCDD were compared with *ad libitum* fed control animals and pair-fed animals. Serum and urine levels of the four metabolites were assayed on days 0, 3, 6, 9 and 12.

Following TCDD administration, significant increases in the four metabolites present in serum and urine were observed at all time points. For example, on day 6 post-treatment malondialdehyde, formaldehyde, acetaldehyde and acetone increased approximately 2.6-, 2.5-, 2.4- and 6.9-fold in serum, respectively, and 3.0-, 2.3-, 3.8- and 3.7-fold in urine, respectively. Increases were also observed in the serum and urine levels of the four metabolites in pair-fed animals relative to the *ad libitum* fed control animals. However, the increases in the serum and urine levels of the four metabolites was significantly greater for TCDD animals as compared to the pair-fed control animals at most time points. When the serum levels of malondialdehyde as determined by HPLC were compared with the results obtained by the thiobarbituric

acid (TBA) colorimetric method, similar time courses were observed although higher results were obtained for the less specific TBA method. The results clearly demonstrate that TCDD causes markedly elevated serum and urine levels of four specific products associated with lipid metabolism.

V. EFFECTS OF CARBON TETRACHLORIDE, MENADIONE, PARAQUAT, AND ALACHLOR ON THE URINARY EXCRETION OF MALONDIALDEHYDE (MDA), FORMALDEHYDE (FA), ACETALDEHYDE (ACT), AND ACETONE (ACON) IN RATS

Excretion of the lipid products FA, MDA, ACT and ACON were simultaneously identified and quantitated in the urine of female Sprague-Dawley rats by the methods described above following the administration of a single, oral, acutely toxic dose of carbon tetrachloride (2.5 ml/kg), menadione (60 mg/kg), paraquat (75 mg/kg), and alachlor (800 mg/kg). These doses are approximately 50% of the LD₅₀s for the four xenobiotics.

Urinary excretion of the four lipid metabolites increased relative to control animals following treatment with all xenobiotics. Over the 48 hours of the study, the greatest increases in the excretion of MDA, ACT and ACON occurred after paraquat administration, with increases of approximately 2.7-, 4.3-, and 11.0-fold, respectively. The greatest increase in the excretion of FA occurred 24 hrs after alachlor administration, with a 15.5-fold increase relative to control values.

The results demonstrate that the methods which have been developed provide an effective technique which has a wide-spread applicability as an effective biomarker for investigating altered lipid metabolism following exposure to a wide range of structurally dissimilar environmental pollutants/xenobiotics. Carbon tetrachloride is a model alkylating agent that does not induce glutathione depletion while paraquat and menadione are redox cycling compounds. Alachlor is a halogenated herbicide which undergoes extensive metabolism, and the marked increase in FA excretion may be due to the formation of FA as a metabolite of the alachlor.

VI. IN VITRO INDUCTION OF REACTIVE OXYGEN SPECIES BY TCDD, ENDRIN AND LINDANE IN RAT PERITONEAL MACROPHAGES, AND HEPATIC MITOCHONDRIA AND MICROSOMES

The ability to induce an oxidative stress by PCH has been well demonstrated in our laboratories. However, the source of the reactive oxygen species is unclear. One of our specific aims was the comparison of the abilities of selected PCH to induce the formation of reactive oxygen species in isolated tissues as rat peritoneal macrophages, and hepatic mitochondria and microsomes.

Hepatic mitochondria and microsomes as well as peritoneal macrophages from female Sprague-Dawley rats were incubated for up to 30 min at 37 °C in the presence of 0-200 ng/ml 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), endrin (2,3,3,4,10,10-hexachloro-6,7-epoxy-1,4- α ,5,6,7,8,8 α -octahydroendo,endo-1,4:5,8-

dimethanonaphthalene) and lindane (hexachlorocyclohexane). Production of reactive oxygen species was determined by chemiluminescence and cytochrome c reduction, while potential tissue damage was assessed by alterations in membrane fluidity. Chemiluminescence, a sensitive but nonspecific measure of free radical generation, increased 40-70% when macrophages (3×10^6 cells/ml), mitochondria and microsomes (1 mg/ml) were incubated with the three polyhalogenated cyclic hydrocarbons (PCH).

Maximum increases in chemiluminescence occurred within 5-10 min of incubation and persisted for over 30 min. The cytochrome c reduction assay is most specific for superoxide anion production. When hepatic mitochondria were incubated with endrin and lindane for 15 min at 100 ng/ml, increases in cytochrome c reduction of 6.5- and 7.5-fold occurred, respectively, while when microsomes were incubated with these same two PCH increases in cytochrome c reduction of 8.6- and 11.6-fold occurred, respectively. When mitochondria, microsomes and macrophages were incubated with TCDD under identical conditions, small increases in superoxide anion production were detected.

Changes in microsomal membrane fluidity were determined spectrofluorometrically following incubation with the three PCH using diphenyl-1,3,5-hexatriene as the fluorescent probe. TCDD, endrin and lindane enhanced microsomal membrane apparent microviscosity by 2.3-, 2.1-, and 2.5-fold, respectively, indicating a significant decrease in membrane fluidity. The results clearly indicate that the three PCH induce free radical formation, but superoxide anion production is enhanced more in the presence of endrin and lindane than with TCDD.

VII. PRODUCTION OF REACTIVE OXYGEN SPECIES BY PERITONEAL MACROPHAGES AND HEPATIC MITOCHONDRIA AND MICROSOMES FROM ENDRIN-TREATED RATS

The above in vitro studies have indicated that incubation of rat peritoneal macrophages, and hepatic mitochondria and microsomes results in the formation of reactive oxygen species. We have therefore assessed the ability of one of these PCH, namely endrin, to induce formation of reactive oxygen species when administered directly to rats.

Endrin was administered in corn oil as a single oral dose of 4.5 mg/kg. The effects of endrin on hepatic mitochondrial and microsomal lipid peroxidation and membrane fluidity as well as the incidence of hepatic nuclear DNA damage were also examined. Twenty-four hrs after endrin administration, significant increases in the production of chemiluminescence by the three tissue fractions were observed. Furthermore, peritoneal macrophages from endrin treated animals resulted in 3.0- and 2.8-fold increases in cytochrome c and iodonitrotetrazolium (INT) reduction, indicating enhanced production of superoxide anion. Endrin administration also resulted in significant increases in lipid peroxidation of mitochondrial and microsomal membranes as well as decreases in the fluidity of these two membranous fractions. A significant increase in hepatic nuclear DNA single strand breaks also occurred in

response to endrin administration. The results indicate that macrophage, mitochondria and microsomes produce reactive oxygen species following endrin administration, and these reactive oxygen species may contribute to the toxic manifestations of endrin.

VIII. ENDRIN-INDUCED PRODUCTION OF NITRIC OXIDE BY RAT PERITONEAL MACROPHAGES

Nitric oxide has been shown to be an important cellular transmitter in many biological systems. Kupffer cells, bone marrow and wound macrophages have the capacity to release nitric oxide as nitrite. The synthesis of nitric oxide by macrophages produce cytotoxic and cytostatic effects. The formation of nitric oxide is an indicator of macrophage activation and the potential production of an oxidative stress. Therefore, the effect of oral endrin administration to rats on the production of nitric oxide (NO) by peritoneal macrophages was investigated. Nitric oxide formation was measured as nitrite. Endrin (4.5 mg/kg) enhanced the secretion of NO by approximately 300%. The effect of endrin on NO formation was both dose- and time-dependent. Ellagic acid, which has been shown to be a potent antioxidant, inhibited the elevation of NO production induced by endrin. These results suggest that the toxicity of endrin may at least in part be due to the production of an oxidative stress.

IX. ENDRIN-INDUCED INCREASES IN HEPATIC LIPID PEROXIDATION, MEMBRANE MICROVISCOSITY AND DNA DAMAGE IN RATS

The above studies have extensively documented that PCH induce an oxidative stress which may contribute to the toxic manifestations of these xenobiotics. In order to further assess the possible role of oxidative stress in the toxicity of the prototypical PCH endrin, the dose- and time-dependent effects of endrin on hepatic lipid peroxidation, membrane microviscosity and DNA damage in rats were examined. Rats were treated with 0, 3.0, 4.5, or 6.0 mg endrin/kg as a single oral dose in corn oil, and the animals were killed 0, 12, 24, 48 or 72 hrs post-treatment.

Dose-dependent increases in hepatic mitochondrial and microsomal lipid peroxidation and microviscosity as well as nuclear DNA single strand breaks were observed as early as 12 hrs post-treatment. Maximum increases in these three parameters occurred 24 hrs after endrin administration at all three doses. While the incidence in DNA damage decreased with time after 24 hrs, the incidence of lipid peroxidation and microviscosity of microsomal and mitochondrial membranes remained relatively constant. Dose- and time-dependent increases in liver and spleen weight/body weight ratios with decreases in thymus weight/body weight ratios were observed. The data indicate that endrin administration induces hepatic lipid peroxidation which may be responsible for the increased membrane microviscosity as a result of membrane damage as well as enhanced DNA damage.

X. EFFECT OF ENDRIN ON THE HEPATIC DISTRIBUTION OF IRON AND CALCIUM IN FEMALE RATS

A possible role between the cellular distribution of iron and the toxicity of PCH which induce an oxidative stress may exist. The role of iron in the formation of reactive oxygen species is well known. Furthermore, the probable role of calcium ions in toxic cell injury has attracted extensive interest. For example, evidence indicates that calcium play an important role in cell killing in the immune system. Sustained increases in intracellular calcium can activate cytotoxic mechanisms associated with irreversible cell injury which ultimately lead to cell death. The perturbation of calcium homeostasis in toxicant-induced liver injury may be due to permeability changes in plasma membranes or altered intracellular calcium distribution. In our studies, we have used endrin as a prototypical PCH, and have examined its effects on iron and calcium distribution in hepatic mitochondria, microsomes and nuclei of rats as a function of dose and time. The effect of vitamin E succinate on endrin-induced alterations in iron and calcium homeostasis was also assessed. If vitamin E succinate can prevent the effects of endrin, the use of this antioxidant further supports the role of oxidative stress and reactive oxygen species in the toxicity of endrin and therefore presumably other PCH.

Endrin in corn oil was administered orally to rats in single doses of 0, 3, 4.5, or 6 mg/kg, and the animals were killed at 0, 12, 24, 48, or 72 hrs post-treatment. Iron and calcium were determined by atomic absorption spectroscopy. The administration of endrin increased the iron content of microsomes and nuclei. Significant increases occurred in the calcium content of mitochondria, microsomes, and nuclei. Thus, the results indicate that with respect to the subcellular distribution of iron and calcium, endrin produces differential effects.

Vitamin E succinate administration partially prevented the endrin-induced hepatic alterations in iron and calcium homeostasis. Endrin also produced dose- and time-dependent increases in the liver and spleen weight/body weight ratios, while decreasing the thymus weight/body weight ratios. The altered distribution of calcium and iron may contribute to the broad range of effects of endrin.

XI. PROTECTIVE EFFECTS OF ANTIOXIDANTS AGAINST ENDRIN-INDUCED HEPATIC LIPID PEROXIDATION, DNA DAMAGE, AND EXCRETION OF URINARY LIPID METABOLITES

If oxidative stress plays a pivotal role in endrin-induced hepatic and neurologic toxicity, antioxidants/free radical scavengers should attenuate the toxic manifestations of endrin. Therefore, the effects of the antioxidants vitamin E succinate and ellagic acid have been examined on hepatic lipid peroxidation, DNA single strand breaks (SSB), and the excretion of urinary lipid metabolites following an acute dose of endrin (4.5 mg/kg).

Groups of rats were pretreated with 100 mg/kg vitamin E succinate for three days followed by 40 mg/kg on day 4, or 6.0 mg ellagic acid/kg for three days orally

followed by 3.0 mg/kg on day 4 or the vehicle. Endrin was administered orally on day 4 two hrs after treatment with the antioxidant. All animals were killed 24 hrs after endrin administration.

Vitamin E succinate pretreatment decreased the endrin-induced increase in hepatic mitochondrial and microsomal lipid peroxidation by approximately 60% and 40%, respectively. Ellagic acid pretreatment reduced the endrin-induced increase in mitochondrial and microsomal lipid peroxidation by approximately 76% and 79%, respectively. Both vitamin E succinate and ellagic acid alone produced small but nonsignificant decreases in hepatic mitochondrial and microsomal lipid peroxidation.

A 3.5-fold increase in the incidence of hepatic nuclear DNA SSB was observed 24 hrs after endrin administration. Pretreatment of rats with vitamin E succinate, vitamin E, and ellagic acid decreased endrin-induced DNA-SSB by approximately 47%, 22%, and 21%, respectively.

Pretreatment of rats with vitamin E succinate decreased the endrin-induced increase in the urinary excretion of MDA, ACT, FA, and ACON by approximately 68%, 65%, 70%, and 55%, respectively. Ellagic acid pretreatment prior to endrin administration decreased the excretion of these same four lipid metabolites by 86%, 90%, 87%, and 73%, respectively. Thus, the results indicate that antioxidants can attenuate the biochemical consequences of endrin-induced oxidative stress, although the effects on DNA-SSB were less pronounced than on lipid peroxidation, which may be associated with compartmentation and antioxidant distribution.

XII. COMPARATIVE STUDIES ON LIPID PEROXIDATION AND DNA-SINGLE STRAND BREAKS INDUCED BY LINDANE, DDT, CHLORDANE AND ENDRIN IN RATS

A wide variety of structurally dissimilar polyhalogenated cyclic hydrocarbons (PCH) produce similar toxic effects. Molecular mechanisms involved in the production of these toxic manifestations are not well understood. The organochlorine insecticides are polyhalogenated hydrocarbons which include chlorinated ethane and cyclodiene derivatives, and hexachlorocyclohexanes, of which DDT, endrin/chlordane and lindane are excellent examples, respectively.

In order to determine whether reactive oxygen species may be involved in the toxicities produced by these xenobiotics, we have examined the time-dependent effects of lindane (30 mg/kg), DDT (40 mg/kg), chlordane (120 mg/kg), and endrin (4.5 mg/kg) on the production of hepatic mitochondrial and microsomal lipid peroxidation and DNA single strand breaks, two indices of oxidative stress.

All four xenobiotics resulted in significant increases in hepatic lipid peroxidation and DNA damage. Earliest (6 hr) increases in both lipid peroxidation and DNA damage were observed following lindane administration. Time-dependent increases in both parameters were observed following endrin administration. Maximum increases in DNA single strand breaks of 2.8- and 2.5-fold were observed 12 hr after DDT and

chlordane administration, respectively, while a 4.5-fold increase was observed 24 hr after endrin administration.

The results clearly demonstrate that the four structurally dissimilar polyhalogenated hydrocarbons produce oxidative tissue damage which may contribute to the toxic manifestations of these xenobiotics. Differences in the responses may reflect dissimilar toxicokinetic properties.

XIII. EXCRETION OF MALONDIALDEHYDE, FORMALDEHYDE, ACETALDEHYDE, ACETONE AND METHYL ETHYL KETONE IN THE URINE OF RATS GIVEN AN ACUTE DOSE OF MALONDIALDEHYDE

Malondialdehyde (MDA) has been extensively used as an index of lipid peroxidation. It is a product of the cyclooxygenase reaction in prostaglandin metabolism and the result of oxidation of polyunsaturated fatty acids with three or more double bonds. MDA also occurs in food products due to the decomposition of polyunsaturated fatty acids. Interest in MDA arises from the fact that it reacts readily with the functional groups of many cellular compounds including amino acid groups of proteins and nucleic acid bases, nitrogenous bases of phospholipids, and the thiol groups of sulfhydryl containing compounds.

The present study investigated the urinary excretion of the lipid metabolites FA, ACT, and ACON as well as MDA and its possible metabolites in response to an acute dose of MDA. The studies were also conducted to provide insight into the metabolic relationships between various urinary excretion products.

Female Sprague-Dawley rats received a single oral dose of MDA (158 mg/kg) in corn oil. Urine samples were collected as previously described. During the first 12 hours following administration of MDA, increases in the urinary excretion of MDA and ACT of approximately 192- and 70-fold, respectively, were observed. The urinary excretion of both MDA and ACT decreased thereafter. An increase in FA excretion was observed only 12-24 hrs after MDA administration. A significant decrease in ACON excretion relative to control values was observed 12-48 hrs after MDA treatment.

Two new peaks were present in the HPLC chromatograms of urine samples 0-24 hrs after MDA administration. The two peaks were shown to be the syn and anti isomers of methyl ethyl ketone (MEK). After MDA administration, MEK excretion increased from 0 to approximately 490 nmoles/kg in the 0-12 hr urine samples and decreased to 14% of this value in the 12-24 hr samples. Beyond 24 hrs after MDA administration, no further excretion of MEK was observed.

Several possible sources exist for the MEK. MEK has been shown to be formed from the 4-carbon compound butane in mice, presumably via 2-butanol, while rat microsomal preparations have been shown to metabolize 2-butanol to MEK. Alternately, MEK might be formed from the condensation of two 2-carbon fragments arising from the metabolism of MDA or MDA-altered lipid and/or carbohydrate metabolism.